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PII: S1056-8719(16)30057-0
DOI: doi: 10.1016/j.vascn.2016.06.001
Reference: JPM 6369

To appear in: *Journal of Pharmacological and Toxicological Methods*

Received date: 22 November 2015
Revised date: 13 January 2016
Accepted date: 1 June 2016

Please cite this article as: Authier, S., Arezzo, J., Delatte, M.S., Kallman, M.-J., Markgraf, C., Paquette, D., Pugsley, M.K., Ratcliffe, S., Redfern, W.S., Stevens, J., Valentin, J.-P., Vargas, H.M. & Curtis, M.J., Safety pharmacology investigations on the nervous system: An industry survey, *Journal of Pharmacological and Toxicological Methods* (2016), doi: 10.1016/j.vascn.2016.06.001

Safety Pharmacology Investigations on the Nervous System: An Industry Survey

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Abstract:

The Safety Pharmacology Society (SPS) conducted an industry survey in 2015 to identify industry practices as they relate to central, peripheral and autonomic nervous system ('CNS') drug safety testing. One hundred fifty-eight (158) participants from Asia (16%), Europe (20%) and North America (56%) responded to the survey. 52% of participants were from pharmaceutical companies (>1,000 employees). Oncology (67%) and neurology/psychiatry (66%) were the most frequent target indications pursued by companies followed by inflammation (48%), cardiovascular (43%), metabolic (39%), infectious (37%), orphan (32%) and respiratory (29%) diseases. Seizures (67% of participants), gait abnormalities (67%), tremors (65%), emesis (56%), sedation (52%) and salivation (47%) were the most commonly encountered CNS issues in pre-clinical drug development while headache (65%), emesis/nausea (60%), fatigue (51%) and dizziness (49%) were the most frequent issues encountered in Phase I clinical trials. 54% of respondents reported that a standard battery of tests applied to screen drug candidates was the approach most commonly used to address non-clinical CNS safety testing. A minority (14% of all participants) reported using electroencephalography (EEG) screening prior to animal inclusion on toxicology studies. The most frequent group size was n=8 for functional observation battery (FOB), polysomnography and seizure liability studies. FOB evaluations were conducted in a dedicated room (78%) by blinded personnel (66%) with control for circadian cycle (55%) effects (e.g., dosing at a standardized time; balancing time of day across treatment groups). The rat was reported as the most common species used for seizure liability, nerve conduction and drug-abuse liability testing.

1. Introduction

With 41 new FDA approved drugs, 2014 was a greater than average year for the pharmaceutical and biotechnology industry (Anon. 2015). This article focuses on CNS drug safety testing, the dynamics between development and Safety Pharmacology methods and strategic approaches that have been taken to garner marketing authorization of a new molecular entity (NME) under New Drug Applications (NDAs) or as new therapeutic biologic under a Biologics License Application (BLA). The aging population has benefited from a moderate but progressive increase in drug development efforts for neurological indications (Melnikova, 2010) and the CNS drug market is forecast to grow to $81.8 billion in 2015 (BCC Research, 2010). Despite this growth to become the 2nd most important therapeutic indication in drug development, CNS indications present a challenge since CNS drugs are reported to take longer to get to market and their attrition rate is greater than other therapeutic indications (Palmer & Alavijeh, 2012).

Market size and level of unmet need determine the clinical importance of drug development within a given therapeutic area which, in turn, reflects upon non-clinical professional disciplines such as Safety Pharmacology (SP). In general terms, although drug approval rates appear to have increased, drug attrition remains a major hurdle in pharmaceutical development (Moreno & Pearson, 2013; Hay et al., 2014; Cook et al., 2014; Waring et al., 2015). A tendency towards an increased failure rate in Phase 3 clinical trials due to safety issues has been reported over a 5 year period (Arrowsmith & Miller, 2013). Oncology remains the largest therapeutic market, with sales exceeding US$100 billion in 2014 after a double digit increase in the previous year (Mullard, 2015). In oncology, neurological adverse effects remain one of the most frequent cause of drug failure (Valentin & Hammond, 2008; Cook et al., 2014), second only to cardiac adverse effects.

Although non-clinical cardiac SP assessment models and testing strategies have been extensively evaluated (Porsolt et al., 2002; Pugsley et al., 2009; Valentin et al., 2010; Authier et al., 2010; Vargas et al., 2015; Klein & Redfern, 2015), only limited information is available with respect to CNS adverse effect testing strategy and practices. At the more granular level, there are comprehensive reviews of non-clinical CNS models for seizure (Bassett et al., 2014; Easter et al., 2009; Metea et al., 2015; Fonck et al., 2015) and drug abuse liabilities (Kallman, 2015; Moser et al., 2010; Porsolt et al., 2002' O'Connor et al., 2011), with consideration of specific screening models for seizure (Authier et al., 2009; Koseki et al., 2014), sleep (Authier et al., 2014) and peripheral nerve function (Zotova & Arezzo, 2013). However, current industry

practices and trends as they relate to the use of these models in drug development are not well characterized (Lindgren et al., 2008).

In view of these considerations, a survey was undertaken by the SPS to evaluate current (defined as during the last 5 years) industry practices for CNS drug SP testing, and the present paper reports the findings.

2. Results

All results are presented as the percentage of total response rate per question, as percentage of total number of scientists that responded to each question or number of responding scientists.

2.1 Study Survey Demographics

Scientists (n=158) from various fields of expertise (Fig. 1A) and location (Fig. 1B) participated in the survey which represented a response rate of 35.3% (158 out of 447 invited). A predominance of participants from North America was inevitable owing to the greater proportion of scientists from this geographical region in the population solicited to take this survey. Participants were distributed between diverse organization types (Fig. 1C) and sizes (Fig. 1D), with more than half from larger companies (>1000 employees). As a result, it is expected that larger companies have contributed multiple respondents from the same organization. Consequently, the results from the survey predominantly reflect practices and perceptions of individuals working in larger institutions. The survey identified some interesting peripheral facts, such as the primary therapeutic areas that were targeted by the companies employing survey participants. Oncology (67%) and neurology/psychiatry (66%) followed by inflammation (48%), cardiovascular (43%), metabolic (38%), infectious (37%), orphan (32%) and respiratory (29%) diseases were the most frequent target therapeutic areas reported (Fig. 1E).

2.2. Drug SP Testing for the CNS: Survey results

Over the last 5 years seizures (67% of participants), gait abnormalities (67%), tremors (65%), emesis (56%), sedation (52%) and salivation (47%) were the most commonly encountered CNS issues in pre-clinical drug development (Figure 2), whereas headache (65%), emesis/nausea (60%), fatigue (51%) and dizziness (49%) were the most frequent issues encountered in Phase I clinical trials (Figure 3). A minority (14% of all participants) reported using pre-study EEG screening of individual animals prior to inclusion in general toxicology studies. A standard battery of tests applied to screen all drug candidates was the approach that best described the strategy used by the company for 54% of participants, whereas 37% selected issue resolution

with tests based on observations from toxicology screening and scientific considerations as the most representative approach used in their company.

In the last 5 years, various CNS assessment technologies and models had been used by participants including gait analysis (45%), elevated plus maze anxiety test (35%), electroretinography (32%), visual acuity (i.e., optomotor reflex) (20%) or pica as a model of nausea (14%). Most participants (82%) had not received regulatory feedback on their CNS safety testing strategy. Of the 18% that had received regulatory feedback, comments on drug abuse liability testing were the most frequent.

2.2.1 Functional Observation Battery (FOB)/Modified Irwin Test

Survey questions treated FOB and Modified Irwin tests together. Scientific literature is available for an in-depth understanding of these assays (Gauvin et al., 1997; Himmel, 2008; Redfern et al., 2005). A majority (79%) of participants had conducted or interpreted FOB/Irwin test results in the last 5 years. From this subgroup, FOB/Irwin tests were most often conducted in rats (96%) and mice (36%) followed by monkeys (35%) and dogs (22%). Only 4% of participants reported experience with FOB in minipigs. A standalone study design was used by a majority (73%) of respondents (Table 2) and half (54%) of the participants had experience of adding the FOB to a toxicology study. When FOB evaluations were conducted after repeated dose administration, the most frequent time points evaluated excluding Day 1 were 7, 14 and 28 days after treatment onset including the last week of treatment. The most frequent FOB group size was n=8 (Figure 4). Respondents reported using study designs that included males only (52%) or males and females (53%) with females only used by 6% of participants. Most participants reported conducting FOB studies under GLP (50%) or both GLP and non-GLP (40%) (Table 3). FOB evaluations were often conducted in a dedicated room (78%), by blinded personnel (66%), with control for circadian cycle (55%) effects (e.g., dosing at a standardized time, or balancing time of day across treatment groups). Three dose levels had been used by two thirds (65%) of participants. A majority of respondents (79%) reported following the modified Irwin test rather than the FOB (Table 4). In contrast, only 9% reported using mazes or cognitive tests. The indication/drug class did not impact the type/extent of FOB/Irwin testing for 64%. For those that reported adjusting the type/extent of FOB/Irwin testing based on indication/drug class (36%), an increased level of testing was triggered by high blood brain barrier (BBB) permeability or CNS indications. Oncology drugs (i.e., ICH S9) and biologics (i.e., ICH S6) were generally viewed as requiring less extensive CNS testing. Statistical analysis was performed on all FOB data by 44% of participants and on selected parameters by 29%. The remainder, 27% did not undertake statistical analysis on FOB data (this probably refers to the incidence data).

2.2.2 Seizure Liability Testing

A majority (55%) of respondents to the survey had conducted or interpreted non-clinical seizure studies within the last 5 years. A minority (19%) of participants routinely conducted seizure studies during the early drug-screening phase of development. Conversely, 76% of participants conducted seizure studies based on results from other studies, and only 10% would terminate development of a compound when seizure liability is present. The therapeutic indication is likely to affect the decision taken in presence of a seizure liability but this was not evaluated in the current survey. A vehicle control (87%) and a positive control (53%) group were included by most participants in seizure liability studies (Table 5) but toxicokinetic (39%), cerebrospinal fluid (17%) and brain tissue (28%) samples were taken only by a minority of participants. The most frequent group size for seizure liability studies was n=8 (Figure 5). Fifteen percent of participants reported conducting all seizure liability assessments under GLP, 43% reported using GLP and non-GLP studies and 43% reported using non-GLP seizure liability studies only (Table 3). This presumably reflects that most seizure liability studies are conducted early in development. As a general principle, one would expect GLP work to be done later in development when candidates with easily identifiable liabilities have been excluded from further development. It appears that there are methods that can be used to identify drug candidates with seizure liabilities early in development before the need to conduct GLP arises (Löscher et al., 1991; Pollack & Shen, 1985). The rat was the most frequently used species with which to test the seizure potential (91%), followed by the mouse (47%) (Table 6). Similarly, rats (87%), dogs (45%), mice (39%) and monkeys (31%) were the most frequently used species when EEG recording was undertaken (Fig. 6). Implanted EEG electrodes (59%) and video monitoring (57%) were the most common methodology used followed by surface/cutaneous EEG (44%) (Table 7). Manual seizure detection (62%) was used more commonly than automated detection (25%). When implanted EEG electrodes were used, the anatomical plane in which the electrodes were implanted was approximately uniformly distributed between subcutaneous (35%), cortical bone (23%), surface of the dura mater (35%) and brain parenchyma (19%). When questioned on interpretation of seizure liability study data, 58% considered that historical data may be used to dismiss an animal with seizure after drug dosing, especially in dogs, and 52% considered the Beagle dog as an appropriate species for seizure liability studies. The presence of clinical signs compatible with seizures needs to be interpreted in relation to drug administration and historical data may be considered to determine if the condition represents a background observation in the model irrespective of treatment or a consequence of dosing. Some participants suggested that pre-study EEG screening was required for Beagle dogs to

ensure suitability for the studies. A majority of participants (73%) considered that a safety margin of 10X to NOAEL for seizures was required while other participants considered 30X preferable, but a safety margin lower than 10X was also reported as acceptable in certain circumstances.

2.2.3 Juvenile CNS Testing

A majority (73%) of respondents had conducted or interpreted non-clinical juvenile CNS testing in the last 5 years. Rats (79%) and mice (32%) were the most common species used for juvenile CNS SP testing followed by non-human primates (24%) and dogs (15%) (Figure 7). This presumably reflects the stage in drug discovery and development in which the testing was undertaken (late stage, and lead candidate, reflected by use of larger mammals or primates). As expected, FOB was the most frequent (91%) juvenile CNS safety assay followed by memory tests (43%), learning tests (40%) and mazes (34%). The Morris water maze was the type most frequently (70%) reported.

2.2.4 Polysomnography (Sleep) Studies

Only 21% of participants had conducted or interpreted polysomnography studies in the previous 5 years. A dose escalation design had been used by 42%, a cross over design by 39%, a repeat dose design had been used by 26%, and 42% of participants reported reusing animals in sleep studies. The dose group size used in these studies varied from 3-12, with n=8 reported to be used most frequently. Smaller group sizes were reported for larger species (i.e., non-human primates). EEG (67%), video monitoring (53%), EMG (43%) and body temperature (43%) were the most frequently used parameters (Table 8). Sleep scoring was undertaken using automated (37%), manual (30%) or both (30%) methodologies.

2.2.5 Peripheral Nerve Conduction Studies

A minority of participants (28%) had conducted or interpreted peripheral nerve conduction studies in the last 5 years. Peripheral nerve conduction was added to a toxicology study by 54% while 49% conducted standalone studies. A repeat dose design (51%) was most frequent but a single dose (19%) or dose escalation (8%) designs were also reported for peripheral nerve function studies. Group size ranged from 6-20 and was reported to typically match the group size for the repeat dose toxicology study. Participants reported conducting peripheral nerve conduction studies under GLP (24%), non-GLP (38%) or both (38%) (Table 3). The rat was the most frequently reported species (72%) (Table 9) used for peripheral nerve conduction assessments. Nerve conduction velocity (68%) and histopathology (62%) were the most frequently selected parameters when conducting peripheral nerve toxicity tests (Table 10).

2.2.6 Drug Abuse Potential Studies

Half (51%) of the participants had conducted or interpreted drug abuse potential studies in the previous 5 years. A typical group size of n=10 was reported for rats and 3-5 for non-rodents. When conducting standalone studies, self-administration (73%), drug withdrawal (47%) and drug discrimination (40%) were the most frequent study types reported by participants. Rat was the most common species (81%) used followed by non-human primate (38%), mouse (19%) and rabbit (6%).

2.2.7 In Vitro Models of CNS Adverse Effects and Other Techniques

In vitro models of CNS adverse effects had been used by a relatively limited proportion of participants. Hippocampal brain slice electrophysiology for seizure liability (30%), neuronal cell lines (27%), *in vitro* blood brain barrier models (20%), neuronal/glial co-culture (17%) and hippocampal brain slice electrophysiology for long-term potentiation (17%) were the most frequently reported models (Table 11). The larval zebrafish seizure assay (15%), stem cell derived neurons (14%) and brain slice electrophysiology studies using other brain regions (13%) were less commonly used (Table 11).

3. Discussion

The current survey aimed to identify current strategies for non-clinical drug SP testing as they relate to CNS adverse effects. Participants were distributed across four continents and originated from diverse company types and sizes. Oncology and neurology were the most frequent indications for which new therapies were developed. The increasing size of the aging population is projected to double the incidence of CNS diseases by 2050 (Wright et al., 2010; Alzheimer's Association, 2015). Concurrently, mortalities from heart diseases, stroke and prostate cancer are expected to decline (Nowbar et al., 2014; Torre et al., 2015). In view of this, CNS adverse effect liability and testing for liability are likely to become more important since drugs for CNS indications (including psychiatric and neurological indications, as well as drugs targeting brain tumors and metastases) represent the broad class with the greatest risk of CNS adverse effect liability, because by nature these agents are CNS active and/or CNS penetrant.

Seizure, gait abnormalities and tremors were the most frequent CNS issues detected in non-clinical drug SP testing in the last 5 years. The incidence of seizures in the elderly population is estimated to be 10-fold higher than in adults (Elberly et al. 2010, Hauser et al., 1993). Susceptibility to drug induced seizures is generally increased in a population with a history of

seizure. Concomitantly, a majority of individuals over the age of 60 take more than three prescription drugs (Gu et al., 2010) supporting a positive correlation between age and the use of polypharmacy. The increasing incidence of seizures with age combined with the frequent use of prescription drugs in an older population places seizure liability testing in a critical position with respect to the risk for adverse drug reaction (ADR). Potentially fatal ADRs such as arrhythmias and seizure mandate a higher level of risk assessment. Drug induced seizures are important medical events with significant impact to patients and are also considered a serious adverse event owing to the potential for status epilepticus, a life-threatening condition that manifests as continuous or rapidly repeating seizures (Gastaut, 1970; Lowenstein et al., 1998). The incidence of status epilepticus has increased four-fold from 1979 to 2010 (Dham et al., 2014) with age considered as an important factor for mortality. Strategies to evaluate seizure liability represent an essential component of risk identification and management in the development of new therapies.

The FOB/Irwin Test can capture a wide range of drug-induced CNS effects (Redfern et al., 2005) and may be adapted to most laboratory animal species including rats (Himmel, 2008), mice (Irwin, 1968), minipigs (Giarola et al., 2008), dogs (Moscardo et al., 2009) and non-human primates (Gauvin & Baird, 2008; Moscardo et al., 2010; Authier et al., 2012). This survey identified the rat as the most frequently used species for FOB studies followed by mice, non-human primates and dogs in approximately equivalent proportions. The abundant historical data in rats and the predominant use of rats as the rodent species for regulatory toxicology studies justifies its selection by default. Species selection is subjected to a plethora of other factors such as pharmacokinetics, metabolism and receptor affinity as well as genetic and phenotypic homology just to name a few. Most participants reported conducting FOB evaluations as standalone studies with three dose levels. Half of the participants (54%) from the current survey reported experience with a design in which a FOB was added to toxicology studies. In a previous industry survey conducted in 2012 by the SPS, 34% of participants had experience with inclusion of FOB in toxicology studies (Authier et al., 2013), possibly indicating increasing experience with the study designs and/or increasing development of biotherapeutic agents over the last three years. When conducted by experienced groups, FOB/Irwin Test assessments were shown to be robust to identify CNS drug effects with known CNS-active agents (Porsolt et al., 2002; Moscardo et al., 2007; Redfern et al., 2005; Ewart et al., 2013), justifying the addition of CNS endpoints in toxicology studies, useful in a 3R's context (Redfern et al., 2013; Redfern, 2015). A wide range of group sizes was reported (i.e. n=3 to 30), but a majority of respondents

used a design with n= 6 or n=8. FOB assessments conducted during early drug discovery are likely to be undertaken as non-GLP while evaluations done during clinical trial enabling studies will typically be completed according to GLP. Only a minority (12%) of participants indicated that FOB assessments were done only non-GLP, but a majority (90%) indicated that FOB evaluations were done under GLP only or both non-GLP and GLP.

Statistical analysis on FOB data appears to be done much less (44%) frequently than one might anticipate. Although it may be perceived that the observational/qualitative nature of most variables makes analysis challenging, whether statistical analysis is permissible or not is primarily dependent on experimental design: adequate group sizes, randomization and blinding, in particular (Curtis & Abernethy, 2015; Curtis et al., 2015). Various established approaches may be used (Moscardo et al., 2007; Markgraf et al., 2010) to identify drug effects in FOB studies. Results from the current survey confirm that a majority of participants undertake statistical analysis (combined 74%), at least for some parameters. For example, Redfern et al. (2005) and Ewart et al (2013) performed statistical analysis of their FOB continuous data and counts, but used a rule-based approach for their incidence data. In simple terms, incidence of an observed parameter was considered noteworthy if it occurred in 50% of the treatment group, the reason being that statistical significance of $P<0.05$ for group sizes of 6 (using Fisher's Exact Test) requires 5/6 rats to exhibit the effect. Setting a lower threshold than this (ie, 3/6) is arguably prudent when in the business of hazard detection. Statistical analysis is more appropriate when using a larger group size and when ranking the severity of the observed parameters, but even so, when Markgraf et al. (2010) compared two nonparametric statistical tests with a qualitative assessment of ranked observations and group sizes of 8, all three methods of analysis provided similar outcomes.

Fifty-two (52) drugs for pediatric indications were approved by the FDA in the last decade (www.centerwatch.com; Accessed on September 23, 2015), representing an important area for pharmaceutical research. A majority of respondents (73%) had experience with juvenile CNS testing and the FOB in young rats was reported as the most frequent assay used (91%). Pharmacological effects identified during FOB may differ between adult and juvenile animals (Himmel, 2008), justifying careful designs supported by regulatory guidance documents (EMEA, 2008; FDA, 2006) to investigate possible neurotoxicity in a younger population when applicable.

A wide range of approved drugs are associated with drug induced sleep disturbances such as insomnia, including beta-blockers (Chang et al., 2013; Moser, 1979), corticosteroids (Ciriaco et al., 2013), selective serotonin-reuptake inhibitors (SSRI) (Asnis, et al., 1999) or acetylcholinesterase inhibitors (Cooke et al., 2006). Polysomnography is a well-established technique for sleep disturbance assessment and is well established in laboratory animal species commonly used for non-clinical drug SP testing including rodents (Gotter et al., 2013), dogs and non-human primates (Authier et al., 2014).

The current survey highlights a high diversity in study designs and methodologies reflecting differences in therapeutic target and stage in the pipeline of drug development. For example, the impact of drug-induced sleep disturbance on drug success depends on the indication since more severe conditions (e.g., oncology) are considered less sensitivity to a potential change in sleep architecture given the severity of the disease and the shorter treatment period compared to other chronic conditions typically requiring lifelong therapies (e.g., diabetes, heart diseases, allergies, neurodegenerative diseases). Moreover, the impact of any adverse effect is entirely dependent on the therapeutic indication; for example, if a drug can treat cancer more effectively than any other, but has some adverse effects on sleep, the drug will be used, whereas if the indication is cough, it is not likely (Pugsley et al., 2008). More exhaustive non-clinical polysomnography studies are more likely to be undertaken for the non-life threatening indications for which drug induced sleep disturbances may have a more clinically relevant impact on the patient population. The tolerance for drug-induced undesired effects is recognized to increase with the severity of the disease that is treated (Pugsley et al., 2008).

Peripheral neuropathies are a common limiting factor with oncology chemotherapeutic drugs (Quasthoff & Hartung, 2002; Wolf et al., 2008; Argyriou et al., 2007). Although the characterization of underlying mechanisms (Berg & Parsons et al., 2015; Bobylev et al., 2015; Li et al., 2015) is progressing and pharmacogenomic strategies are emerging (Gambarotta et al., 2014), non-clinical electrophysiology models and behavioral assessments (mechanical allodynia and hyperalgesia) remain a cornerstone to identify drug-induced peripheral neuropathies. The rat is the most frequently used species for peripheral nerve function assessments. Rats were successfully used to identify peripheral neuropathy induced by chemotherapeutic drugs such as carboplatin (Cavaletti et al., 1998), cisplatin (Bianchi et al., 2006), paclitaxel (Cavaletti et al., 1997) and vincristine (Alimoradi et al., 2012). Like any non-clinical model, the translational potential of peripheral nerve conduction tests remains imperfect. This is illustrated by

thalidomide which is associated with peripheral neuropathy in patients (Kocer et al., 2009) but failed to alter sensory nerve conduction velocity in a chronic toxicology study in Beagle dogs (Teo et al., 2000). Although it is often omitted, the reporting of both nerve conduction and histopathology findings should include the location of the points of assessments along a distal-to-proximal gradient. The absence of deficits measured at a relatively proximal site may yield a false negative finding in the presence of a "length-dependent distal axonopathy (Arezzo, J.C., et al., 2011)." This is especially true for sensory neuropathies manifested in the elongated caudal nerve of the rat (Schaumburg et al., 2010).

The current survey suggests that drug abuse liability was amongst the most frequent CNS follow-up SP studies conducted with 51% of the participants that had conducted or interpreted drug abuse potential studies in the last 5 years. As previously reported, drug abuse liability studies are conducted GLP or non-GLP (Moser et al., 2011). Robust scientific literature exists to support the use of rats for regulatory drug abuse liability studies (O'Connor et al., 2011; Hudzik et al., 2013; Teuns et al., 2014) and this was echoed in the current survey in which a large majority (81%) of participants reported using this species. Amongst survey participants with experience in drug abuse liability testing, the three core pieces of behavioral data required in the overall abuse liability analysis (Ator & Griffiths, 2003) were the most frequently used (i.e., self-administration (73%), drug withdrawal (47%) and drug discrimination (40%)). Participants reported a higher frequency of regulatory feedback on drug abuse liability studies when compared to other CNS drug safety testing areas. Drug abuse liability studies are expected as part of the End of Phase 2 package for CNS-active drugs and therefore the high frequency of regulatory feedback is not unexpected. Furthermore, these well-defined studies are reported in the literature (Gauvin et al., 2015) and regulatory guidelines (EMEA, 2006; FDA, 2010), which can be used to support the study design rationale.

4. Conclusion

In conclusion, this industry survey conducted by the Safety Pharmacology Society (SPS) provides a snapshot of the current non-clinical CNS drug SP testing landscape. It confirms a number of well-accepted paradigms but also reveals study design trends for common follow-up models in CNS SP. Functional endpoints in CNS SP should exhibit high sensitivity and high translational value. Future non-clinical CNS industry surveys will reveal whether improvements have been made in this regard. While this industry survey covered frequently used pre-clinical CNS safety testing models, the field is rapidly progressing with emerging considerations and assays such as neuropsychiatric liability, negative affective bias models, intracranial self-

stimulation for anhedonia, microelectrode *in vitro* neuron network screening platforms or integrated safety assessments that take into consideration functional activity on CNS receptors that are identified during *in vitro* receptor profiling. Beyond the current survey, the Safety Pharmacology Society (SPS) plays an important role to unveil industry trends related to innovative functional drug safety testing strategies.

Acknowledgement
The authors wish to thank the Safety Pharmacology Society (SPS) for support in the conduct of this survey.

Conflict of Interest
None of the authors have any conflicts of interest, other than their employment in commercial pharmaceutical companies, academic institutions or contract research organizations. No information is presented in this paper that advocates for or promotes commercial products from any of our organizations.

References

Alimoradi, H., Pourmohammadi, N., Mehr, S.E., Hassanzadeh, G., Hadian, M.R., Sharifzadeh, M., Bakhtiarian, A., & Dehpour, A.R. (2012). Effects of lithium on peripheral neuropathy induced by vincristine in rats. *Acta Medica Iranica*, 50, 373-379.

Alzheimer's Association. (2015). 2015 Alzheimer's disease facts and figures. *Alzheimers Dementia*, 11, 332-384.

Anon (2015).Novel New Drugs 2014 Summary. http://www.fda.gov/downloads/Drugs/DevelopmentApprovalProcess/DrugInnovation/UCM430299.pdf

Arezzo, J.C., Litwak, M, and Zotova, E. Correlation and Dissociation of Electrophysiology and Histopathology in the Assessment of Toxic Neuropathy. Toxicologic Pathology, 39:1 46-51, 2011.

Argyriou, A.A., Polychronopoulos, P., Koutras, A., Xiros, N., Petsas, T., Argyriou, K., Kalofonos, H.P., & Chroni, E. (2007). Clinical and electrophysiological features of peripheral neuropathy induced by administration of cisplatin plus paclitaxel-based chemotherapy. *European Journal of Cancer Care*, 16, 231-237.

Arrowsmith, J., & Miller, P. (2013). Trial watch: phase II and phase III attrition rates 2011-2012. *Nature Review Drug Discovery*, 12, 569.

Asnis, G.M., Chakraburtty, A., DuBoff, E.A., Krystal, A., Londborg, P.D. et al. (1999). Zolpidem for persistent insomnia in SSRI-treated depressed patients. *Journal of Clinical Psychiatry*, 60, 668-676.

Ator, N.A., & Griffiths, R.R. (2003). Principles of drug abuse liability assessment in laboratory animals. *Drug and Alcohol Dependence*, 70, S55−S72.

Authier, S., Bassett, L., Pouliot, M., Rachalski, A., Troncy, E., Paquette, D., & Mongrain, V. (2014). Effects of amphetamine, diazepam and caffeine on polysomnography (EEG, EMG, EOG)-derived variables measured using telemetry in Cynomolgus monkeys. *Journal of Pharmacological & Toxicological Methods*, 70, 86-93.

Authier, S., Bienvenu, J.G., Tavcar, R., Fournier, S., Schneider, A., & Wong, K. (2012). Functional observation battery in nonhuman primates with continuous intracerebral infusion. *Journal of Pharmacological & Toxicological Methods*, 66, 162.

Authier, S., Paquette, D., Gauvin, D., Sammut, V., Fournier, S., Chaurand, F., & Troncy, E. (2009). Video-electroencephalography in conscious non-human primate using radiotelemetry and computerized analysis: refinement of a safety pharmacology model. *Journal of Pharmacological & Toxicological Methods*, 60, 88-93.

Authier, S., Pugsley, M.K., Troncy, E., & Curtis, M.J. (2010). Arrhythmogenic liability screening in cardiovascular safety pharmacology: commonality between non-clinical safety pharmacology and clinical thorough QT (TQT) studies. *Journal of Pharmacological & Toxicological Methods*, 62, 83-88.

Authier, S., Vargas, H.M., Curtis, M.J., Holbrook, M., & Pugsley, M.K. (2013). Safety pharmacology investigations in toxicology studies: an industry survey. *Journal of Pharmacological & Toxicological Methods*, 68, 44-51.

Baird, S. J., Catalano, P. J., Ryan, L. M., and Evans, J. S. (1997). Evaluation of effect profiles: Functional Observational Battery outcomes. *Fundamental and Applied Toxicology:* **40**: 37-51.

Bassett, L., Troncy, E., Pouliot, M., Paquette, D., Ascah, A., & Authier, S. (2014). Telemetry video-electroencephalography (EEG) in rats, dogs and non-human primates: methods in follow-up safety pharmacology seizure liability assessments. *Journal of Pharmacological & Toxicological Methods*, 70, 230-240.

Berg, S.L., & Parsons, D.W. (2015). The pharmacogenomics of vincristine-induced neuropathy: on pins and needles. *Journal of the American Medical Association Oncology*, (in press).

Bianchi, R., Brines, M., Lauria, G., Savino, C., Gilardini, A., Nicolini, G., Rodriguez-Menendez, V. et al. (2006). Protective effect of erythropoietin and its carbamylated derivative in experimental Cisplatin peripheral neurotoxicity. *Clinical Cancer Research*, 12, 2607-2612.

Bobylev, I., Joshi, A.R., Barham, M., Ritter, C., Neiss, W.F., Höke, A., & Lehmann, H.C. (2015). Paclitaxel inhibits mRNA transport in axons. *Neurobiological Disease*, 82, 321-331.

Cavaletti, G., Cavalletti, E., Montaguti, P., Oggioni, N., De Negri, O., & Tredici, G. (1997). Effect on the peripheral nervous system of the short-term intravenous administration of paclitaxel in the rat. *Neurotoxicology*, 18, 137-145.

Cavaletti, G., Fabbrica, D., Minoia, C., Frattola, L., & Tredici, G. (1998). Carboplatin toxic effects on the peripheral nervous system of the rat. *Annals of Oncology*, 9, 443-447.

Chang, C.H., Yang, Y.H., Lin, S.J., Su, J.J., Cheng, C.L., & Lin, L.J. (2013). Risk of insomnia attributable to β-blockers in elderly patients with newly diagnosed hypertension. *Drug Metabolism Pharmacokinetics*, 28, 53-58.

Committee for Human Medicinal Products, European Medicines Agency, London, UK. http://www.emea.eu.int/pdfs/human/swp/16921505en.pdf

Cooke, J.R., Loredo, J.S., Liu, L., Marler, M., Corey-Bloom, J., Fiorentino, L., Harrison, T., & Ancoli-Israel, S. (2006). Acetylcholinesterase inhibitors and sleep architecture in patients with Alzheimer's disease. *Drugs Aging,* 23, 503-511.

Curtis, M.J., Abernethy, D.R. (2015). Revision of instructions to authors for pharmacology research and perspectives: enhancing the quality and transparency of published work. *Pharmacology Research and Perspectives* e00106.

Curtis, M.J., Bond, R.A., Spina, D., Ahluwalia, A., Alexander, S.P.A., Giembycz, M.A., Gilchrist, A., et al. (2015). Experimental design and analysis and their reporting: new guidance for publication in BJP. *British Journal of Pharmacology,* 172, 2671-2674.

Dham, B.S., Hunter, K., & Rincon, F. (2014). The epidemiology of status epilepticus in the United States. *Neurocritical Care*, 20, 476-483.

Easter, A., Bell, M.E., Damewood, J.R. Jr., Redfern, W.S., Valentin, J.P., Winter, M.J., Fonck, C., & Bialecki, R.A. (2009). Approaches to seizure risk assessment in preclinical drug discovery. *Drug Discovery Today*, 14, 876-884.

Elberly, L.E., Leppik, I.E., Svendsen, K.H., et al. (2010). Incident epilepsy/seizure disorder in US nursing homes by predisposing conditions, 2003–2007. *Neurology,* 74, A42.

European Medicines Agency (EMEA) (2006). Guideline on the non-clinical investigation of the dependence potential of medicinal products. EMEA/CHMP/SWP/94227/2004.

European Medicines Agency (EMEA) (2008). Guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for pediatric indications. EMEA/CHMP/ SWP/ 169215/2005.

Ewart, L., Milne, A., Adkins, D., Benjamin, A., Bialecki, R., Chen, Y., Ericsson, A.C., Gardner, S., Grant, C., et al. (2013). A multi-site comparison of in vivo safety pharmacology studies conducted to support ICH S7A & B regulatory submissions. *Journal of Pharmacological & Toxicological Methods*, 68, 30-43.

Fonck, C., Easter, A., Pietras, M.R., & Bialecki, R.A. (2015). CNS Adverse Effects: From Functional Observation Battery/Irwin Tests to Electrophysiology. *Handbook of Experimental Pharmacology*, 229, 83-113.

Food and Drug Administration (FDA) Guidance Document (2006). Nonclinical safety evaluation of pediatric drug products. Food and Drug Administration, U.S. Department of Health and Human Services, Rockville, MD, USA. http://www.fda. gov/cder/guidance/5671fnl.pdf

Food and Drug Administration (FDA) Guidance for Industry (2010). Assessment of Abuse Potential of Drugs. Food and Drug Administration, U.S. Department of Health and Human Services, Rockville, MD, USA.

http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm198650.pdf

Gambarotta, G., Ronchi, G., Friard, O., Galletta, P., Perroteau, I., & Geuna, S. (2014). Identification and validation of suitable housekeeping genes for normalizing quantitative real-time PCR assays in injured peripheral nerves. *PLoS One*, 9(8):e105601.

Gastaut, H. (1970). Clinical and electroencephalographical classification of epileptic seizures. *Epilepsia*, 11, 102-113.

Gauvin, D.V., McComb, M., Code, R., Dalton, J.A., & Baird, T.J. (2015). Abuse liability assessment of hydrocodone under current draft regulatory guidelines. *Journal of Pharmacological & Toxicological Methods*, pii: S1056-8719(15)00050-7. doi: 10.1016/j.vascn.2015.05.003.

Gauvin, D.V., & Baird, T.J. (2008). A functional observational battery in non-human primates for regulatory-required neurobehavioral assessments. *Journal of Pharmacological & Toxicological Methods*, 58, 88-93.

Giarola, A., Moscardo, E., Fasdelli, N., Tontodonati, M., & Dorigatti, R. (2008). Neurobehavioural assessment in pre-clinical species: Rodents, dogs, mini-pigs and monkeys. *Journal of Pharmacological & Toxicological Methods*, 58, 151.

Gotter, A.L., Winrow, C.J., Brunner, J., Garson, S.L., Fox, S.V., Binns, J., Harrell, C.M., Cui, D., Yee, K.L., et al. (2013). The duration of sleep promoting efficacy by dual orexin receptor antagonists is dependent upon receptor occupancy threshold. *BMC Neuroscience,* 14:90.

Gu, Q., Dillon, C.F., & Burt, V.L. (2010). Prescription drug use continues to increase: U.S. prescription drug data for 2007-2008. *NCHS Data Brief*, 42, 1-8.

Hauser, W.A., Annegers, J.F., & Kurland, L.T. (1993). Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935–1984. *Epilepsia.* 34, 453–468.

Hay, M., Thomas, D.W., Craighead, J.L., Economides, C., & Rosenthal, J. (2014). Clinical development success rates for investigational drugs. *Nature Biotechnology*, 32, 40-51.

Himmel, H.M. (2008). Safety pharmacology assessment of central nervous system function in juvenile and adult rats: effects of pharmacological reference compounds. *Journal of Pharmacological & Toxicological Methods*, 58, 129-146.

Hudzik, T.J., Basso, A., Boyce-Rustay, J.M., Bracken, W., Browman, K.E., Drescher, K., Esbenshade, T.A., Loberg, L.I., Lynch, J.J. 3rd, & Brioni, J.D. (2013). Assessment of the abuse liability of ABT-288, a novel histamine H_3 receptor antagonist. *Psychopharmacology*, 228, 187-197.

Irwin, S. (1968). Comprehensive observational assessment: A systematic quantitative procedure for assessing the behavioural and physiologic state of the mouse. *Psychopharmacology*, 13, 222−257.

Kallman, M.J. (2015). Preclinical Abuse Potential Assessment. *Handbook of Experimental Pharmacology*, 229, 115-30.

Klein, S.K., & Redfern, W,S. (2015). Cardiovascular safety risk assessment for new candidate drugs from functional and pathological data: Conference report. *Journal of Pharmacological & Toxicological Methods*, 76, 1-6.

Kocer, B., Sucak, G., Kuruoglu, R., Aki, Z., Haznedar, R., & Erdogmus, N.I. (2009). Clinical and electrophysiological evaluation of patients with thalidomide-induced neuropathy. *Acta Neurologica*, 109, 120-126.

Koseki, N., Deguchi, J., Yamashita, A., Miyawaki, I., & Funabashi, H. (2014). Establishment of a novel experimental protocol for drug-induced seizure liability screening based on a locomotor activity assay in zebrafish. *Journal of Toxicological Science*, 39, 579-600.

Li, Y., Zhang, H., Kosturakis, A.K., Cassidy, R.M., Zhang, H., Kennamer-Chapman, R.M., Jawad, A.B., Colomand, C.M., Harrison, D.S., & Dougherty, P.M. (2015). MAPK signaling downstream to TLR4 contributes to paclitaxel-induced peripheral neuropathy. *Brain Behavior Immunology*, pii: S0889-1591(15)00154-3.

Lindgren, S., Bass, A.S., Briscoe, R., Bruse, K., Friedrichs, G.S., Kallman, M.J., Markgraf, C., Patmore, L., & Pugsley, M.K. (2008). Benchmarking safety pharmacology regulatory packages and best practice. *Journal of Pharmacological & Toxicological Methods*, 58, 99-109.

Löscher W, Hönack D, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizure models. Epilepsy Res. 1991 Apr;8(3):171-89.

Lowenstein, D.H., & Alldredge, B.K. (1998). Status epilepticus. *New England Journal of Medicine*, 338, 970-976.

Markgraf, C.G., Cirino, M., & Meredith, J. (2010). Comparison of methods for analysis of functional observation battery (FOB) data. *Journal of Pharmacological & Toxicological Methods*, 62, 89-94.

Melnikova, I. (2010). Pain market. *Nature Review Drug Discovery*, 9, 589-590.

Metea, M., Litwak, M., & Arezzo, J. (2015). Assessment of seizure risk in pre-clinical studies: Strengths and limitations of the electroencephalogram (EEG). *Journal of Pharmacological & Toxicological Methods*, pii: S1056-8719(15)00039-8. doi: 10.1016/j.vascn.2015.04.001

Moreno, L., & Pearson, A.D. (2013). How can attrition rates be reduced in cancer drug discovery? *Expert Opinion in Drug Discovery*, 8, 363-368.

Moscardo, E., Fasdelli, N., Giarola, A., Tontodonati, M., & Dorigatti, R. (2009). An optimized neurobehavioural observation battery integrated with the assessment of cardiovascular function in the beagle dog. *Journal of Pharmacological & Toxicological Methods*, 60, 198-209.

Moscardo E, Maurin A, Dorigatti R, Champeroux P, Richard S. An optimized methodology for the neurobehavioural assessment in rodents. J Pharmacol Toxicol Methods. 2007 Sep-Oct;56(2):239-55.

Moscardo, E., McPhie, G., Fasdelli, N., Giarola, A., Tontodonati, M., Dorigatti, R., & Meecham, K. (2010). An integrated cardiovascular and neurobehavioural functional assessment in the conscious telemetered cynomolgus monkey. *Journal of Pharmacological & Toxicological Methods*, 62, 95-106.

Moser, M. (1979). Propranolol and newer antihypertensive drugs in the management of hypertension. *Advances in Cardiology*, 26, 38-43.

Moser, P., Wolinsky, T., Castagné, V., & Duxon, M. (2010). Current approaches and issues in non-clinical evaluation of abuse and dependence. *Journal of Pharmacological & Toxicological Methods*, 63, 160-167.

Mullard, A. (2015). Cancer market hits $100 billion. *Nature Reviews Drug Discovery*, 14, 373.

Nowbar AN, Howard JP, Finegold JA, Asaria P, Francis DP. 2014 global geographic analysis of mortality from ischaemic heart disease by country, age and income: statistics from World Health Organisation and United Nations. Int J Cardiol. 2014 Jun 15;174(2):293-8.

Palmer, A.M., & Alavijeh, M.S. (2012). Translational CNS medicines research. *Drug Discovery Today*, 17, 1068-1078.

Pollack GM, Shen DD. A timed intravenous pentylenetetrazol infusion seizure model for quantitating the anticonvulsant effect of valproic acid in the rat. J Pharmacol Methods. 1985 Apr;13(2):135-46.

Porsolt, R.D., Lemaire, M., Dürmüller, N., & Roux, S. (2002). New perspectives in CNS safety pharmacology. *Fundamentals of Clinical Pharmacology*, 16, 197-207.

Pugsley, M.K., Authier, S., & Curtis, M.J. (2008). Principles of Safety Pharmacology. *British Journal of Pharmacology*, 154, 1382-1399.

Pugsley, M.K., Authier, S., Towart, R., Gallacher, D.J., & Curtis, M.J. (2009). Beyond the safety assessment of drug-mediated changes in the QT interval... what's next? *Journal of Pharmacological & Toxicological Methods*, 60, 24-27.

Quasthoff, S., & Hartung, H.P. (2002). Chemotherapy-induced peripheral neuropathy. *Journal of Neurology*, 249, 9-17.

Redfern WS. (2015) Inclusion of safety pharmacology endpoints in repeat-dose toxicity studies. In *Principles of Safety Pharmacology*, Series: Handbook of Experimental Pharmacology, Vol. 229, pp. 353-381. M.K. Pugsley, M. Curtis (Eds.), Springer.com.

Redfern WS, Ewart LC, Lainée P, Pinches M, Robinson S, Valentin J-P. (2013) Functional Assessments in Repeat-dose Toxicity Studies: The Art of the Possible. *Toxicology Research* **2:** 209-234.

Redfern, W.S., Strang, I., Storey, S., Heys, C., Barnard, C., Lawton, K., Hammond, T.G., & Valentin, J.P. (2005). Spectrum of effects detected in the rat functional observational battery following oral administration of non-CNS targeted compounds. *Journal of Pharmacological & Toxicological Methods*, 52, 77-82.

Schaumburg, H.H., Zotova, E, Raine, C.S., Tar, M., Arezzo, J.C. The caudal nerves: a model system for experimental neuropathies, Journal of the Peripheral Nervous System, 15:128-139, 2010.

Teo, S., Evans, M., Ehrhart, J., Brockman, M., Allen, D., Morgan, M., Stirling, D., & Thomas, S. (2000). Lack of peripheral neuropathy in Beagle dogs after 53 weeks oral administration of thalidomide capsules. *Human Experimental Toxicology*, 19, 615-622.

Teuns, G.B., Geys, H.M., Geuens, S.M., Stinissen, P., & Meert, T.F. (2014). Abuse liability assessment in preclinical drug development: predictivity of a translational approach for abuse liability testing using methylphenidate in four standardized preclinical study models. *Journal of Pharmacological & Toxicological Methods*, 70, 295-309.

Therapeutic Drugs for Central Nervous System Disorders: Technologies and Global Markets, *BCC Research,* (2010) http://www.bccresearch.com/report/drugs-central-nervous-system-disorders-phm068a.html.

Valentin, J.P., Pollard, C., Lainée, P., & Hammond, T. (2010). Value of non-clinical cardiac repolarization assays in supporting the discovery and development of safer medicines. *British Journal of Pharmacology,* 159, 25-33.

Valentin, J.P., & Hammond, T. (2008). Safety and secondary pharmacology: successes, threats, challenges and opportunities. *Journal of Pharmacological & Toxicological Methods*, 58, 77-87.

Vargas, H.M., Bass, A.S., Koerner, J., Matis-Mitchell, S., Pugsley, M.K., Skinner, M., Burnham, M., Bridgland-Taylor, M., Pettit, S., & Valentin, J.P. (2015). Evaluation of drug-induced QT interval prolongation in animal and human studies: a literature review of concordance. *British Journal of Pharmacology*, 172, 402-11.

Waring, M.J., Arrowsmith, J., Leach, A.R., Leeson, P.D., Mandrell, S., Owen, R.M., et al. (2015). An analysis of the attrition of drug candidates from four major pharmaceutical companies. *Nature Review Drug Discovery*, 14, 475-486.

Wolf, S., Barton, D., Kottschade, L., Grothey, A., & Loprinzi, C. (2008). Chemotherapy-induced peripheral neuropathy: prevention and treatment strategies. *European Journal of Cancer*, 44, 1507-1515.

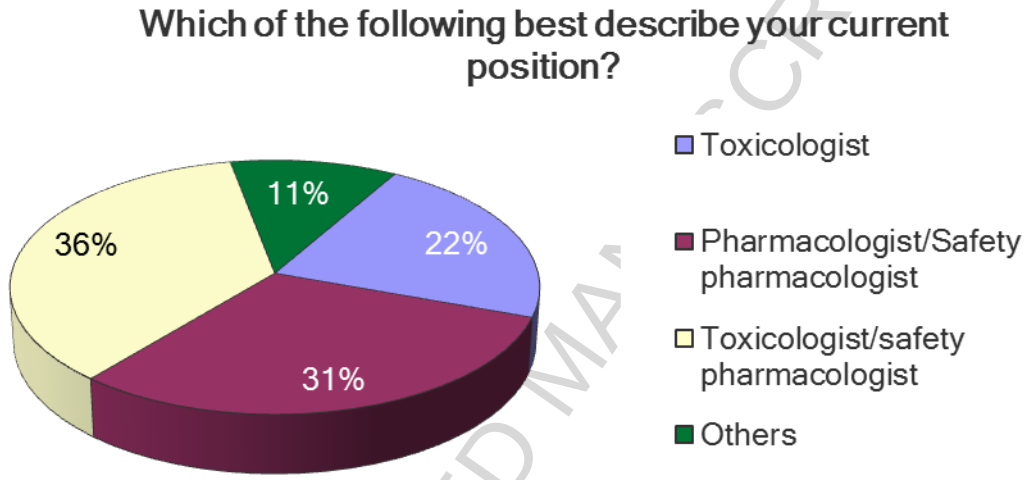
Wright Willis, A., Evanoff, B.A., Lian, M., Criswell, S.R., & Racette, B.A. (2010). Geographic and ethnic variation in Parkinson disease: a population-based study of US Medicare beneficiaries. *Neuroepidemiology*, 34, 143-151.

Zotova, E.G., & Arezzo, J.C. (2013). Non-invasive evaluation of nerve conduction in small diameter fibers in the rat. *Physiology Journal*. 2013: pii: 254789.

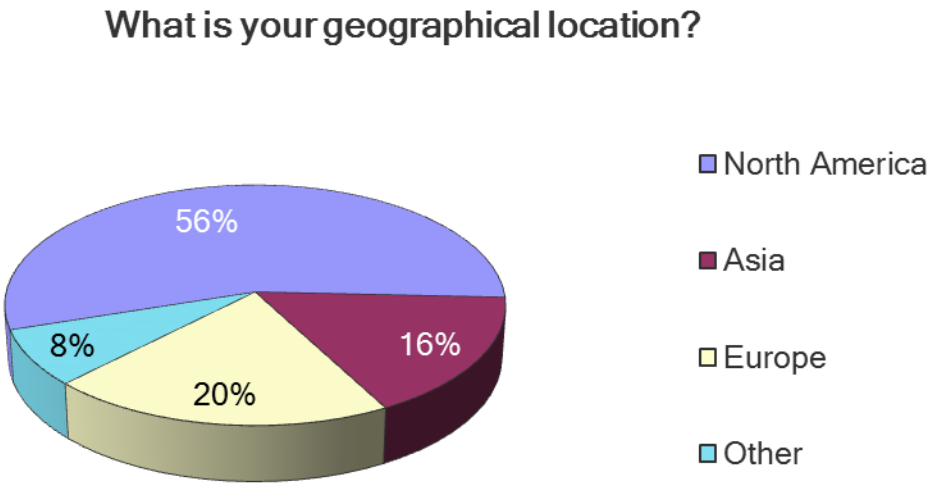
Figures

Figure 1 – Central Nervous System Safety Pharmacology Investigations: Survey Demographics

Panel A



Panel B



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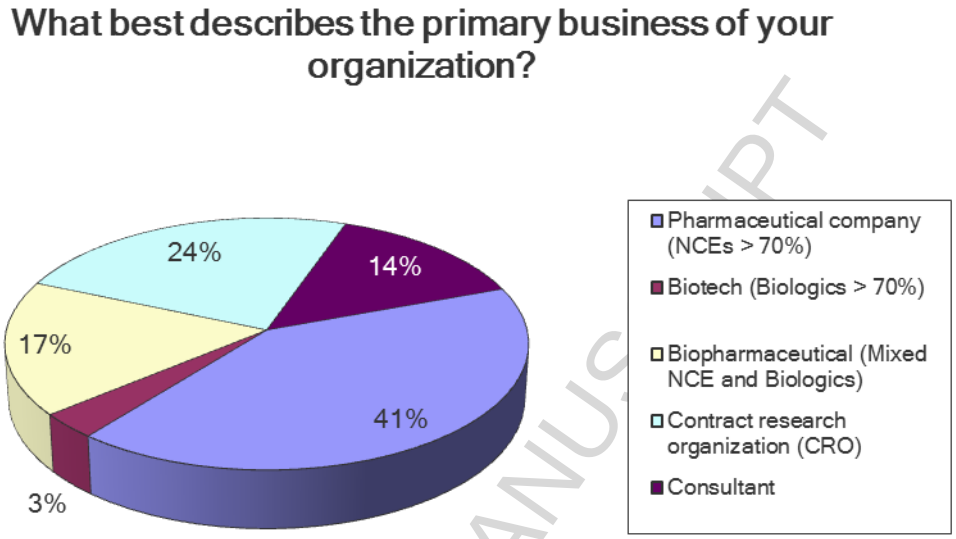
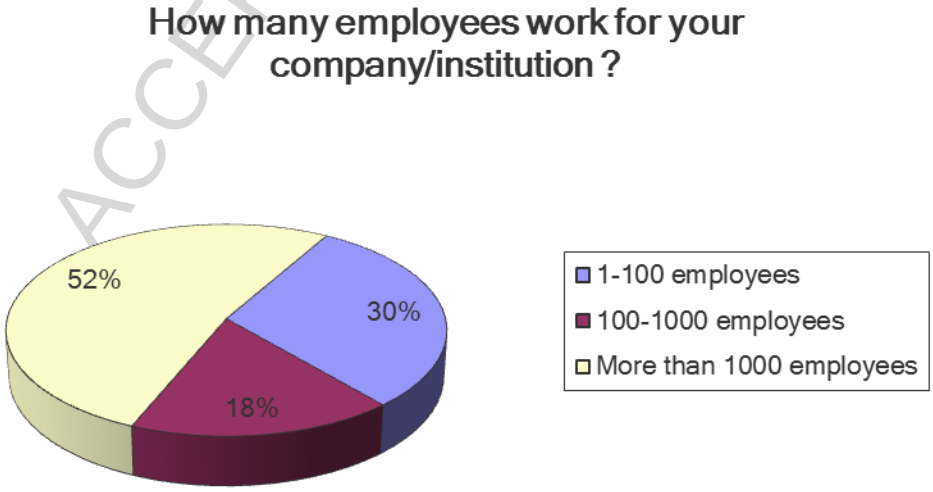


Panel D



Panel E

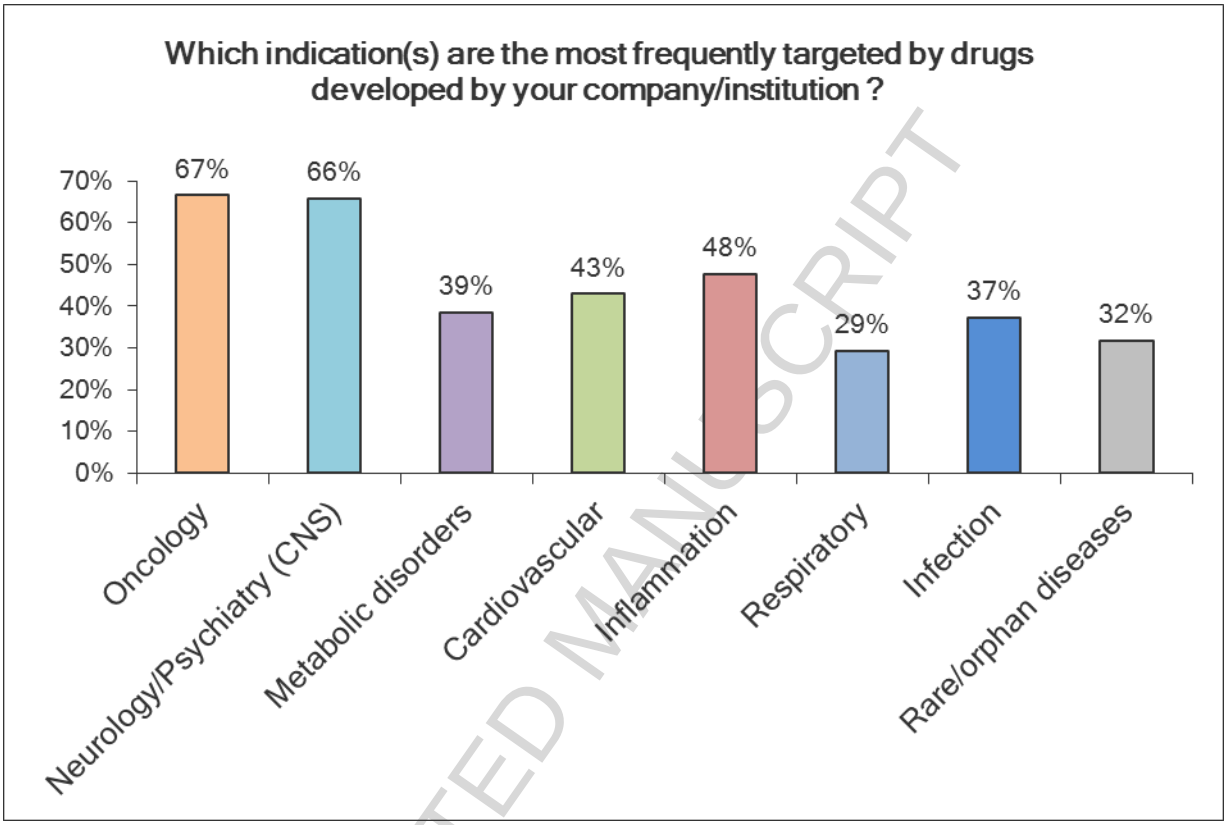


Figure 1 – Panels A-E describe the characteristics of those responding to the survey in terms of expertise (A), geographical location (B), organization affiliation (Pharmaceutical, Biotechnology, Biopharmaceutical, Consultancy), size of the organization (D) and indication most frequently targeted by drugs developed from 2010 to 2015 (E).

Figure 2

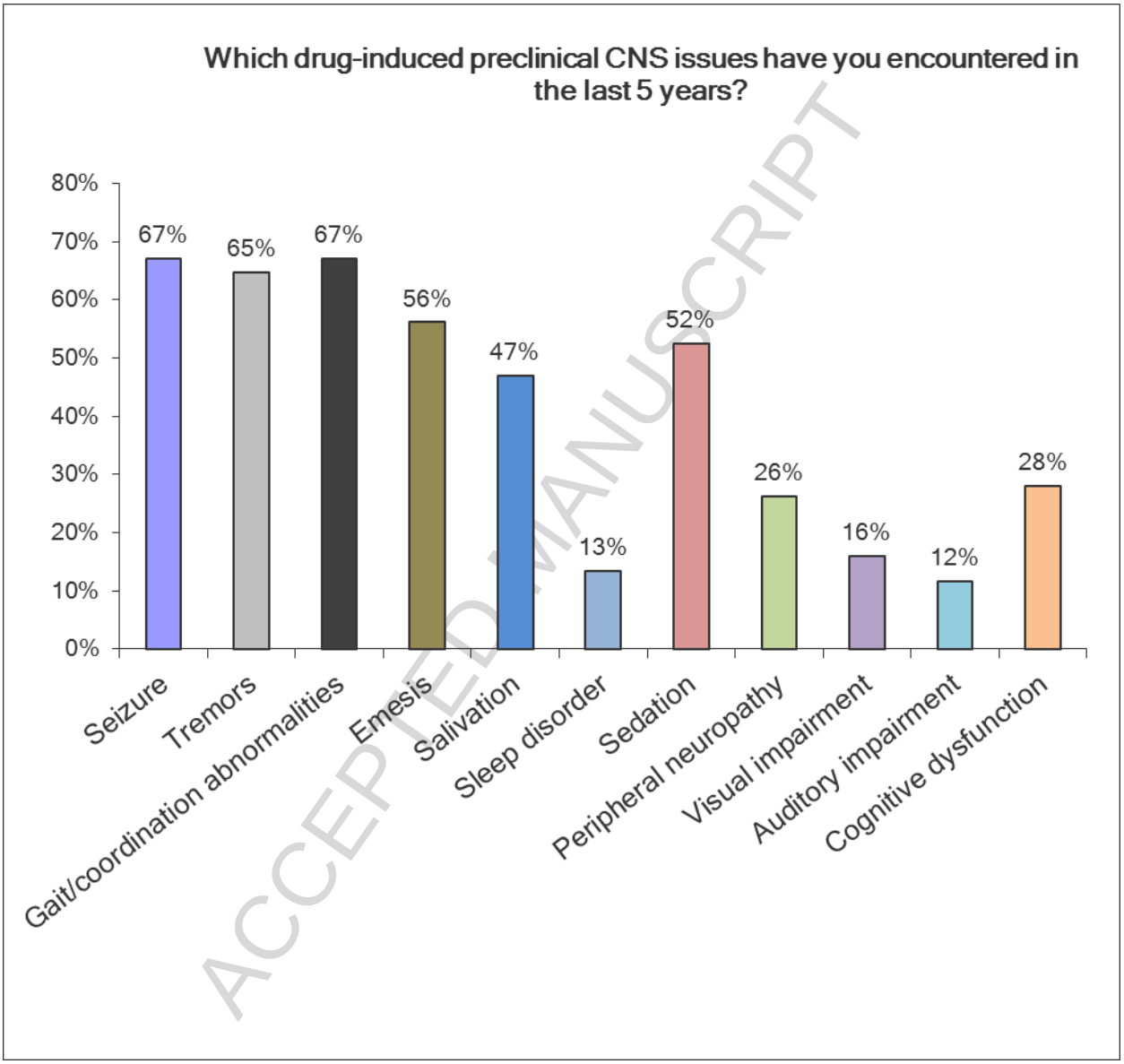


Figure 2: Types of CNS issues encountered in preclinical studies from 2010 to 2015. Emesis was included in the broad list of drug-induced neurological issues as it can originate from CNS or non-CNS etiologies.

Figure 3

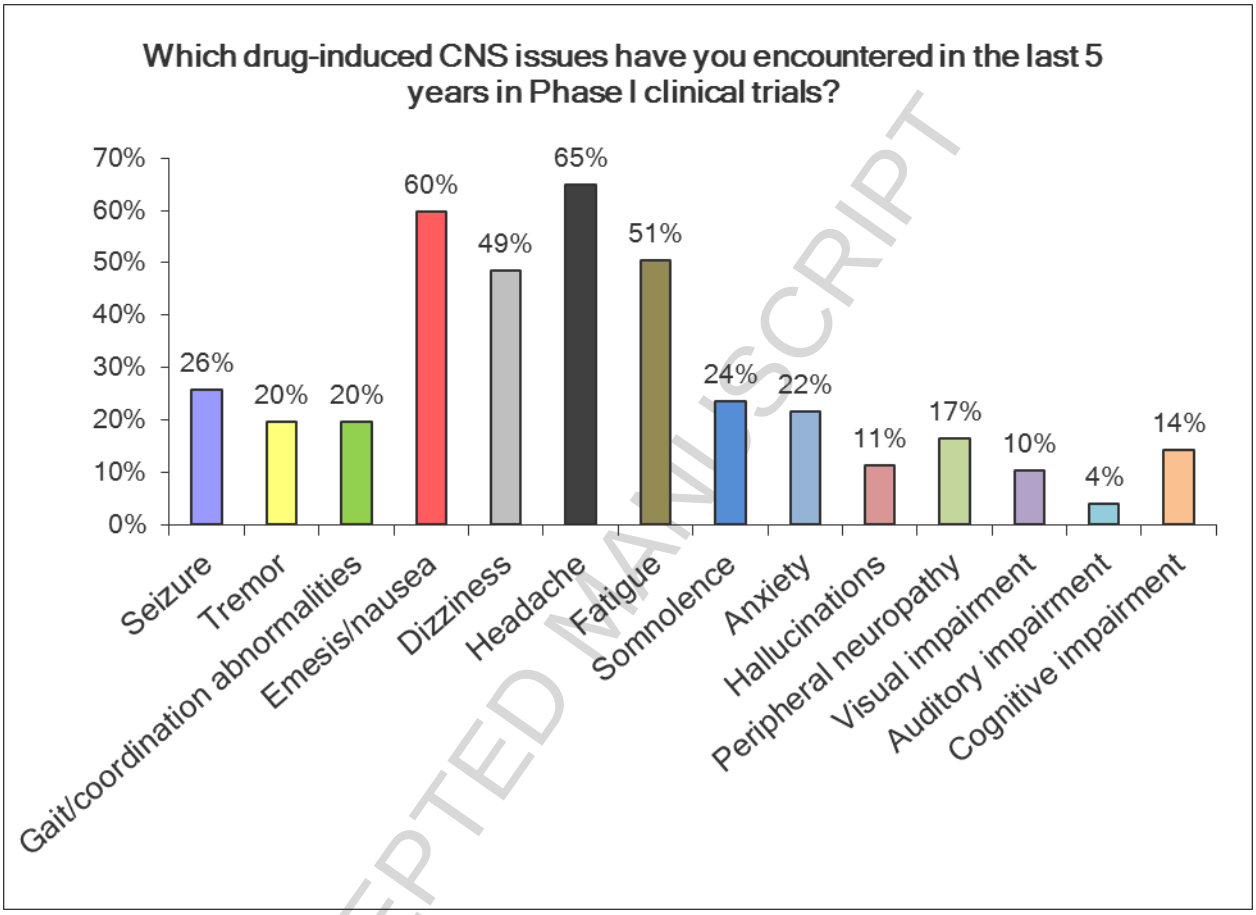


Figure 3: Types of CNS issues encountered in Phase I clinical trials from 2010 to 2015. Although emesis/nausea and fatigue are not typically considered CNS issues from a clinical adverse event terminology perspective, they have been included in this assessment due to the notion that the event reporting requires the element of perception that is under CNS control.

Figure 4

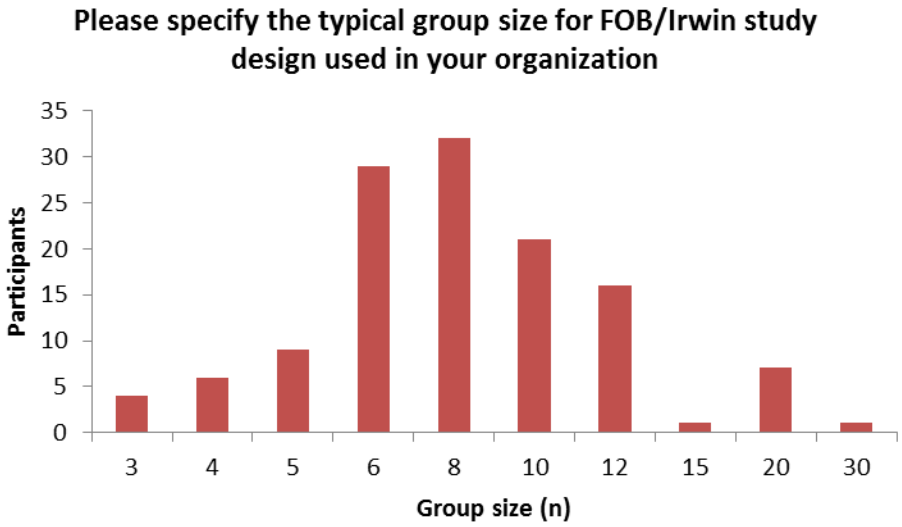


Figure 4: Typical group size in functional observation battery (FOB) studies.

Figure 5.

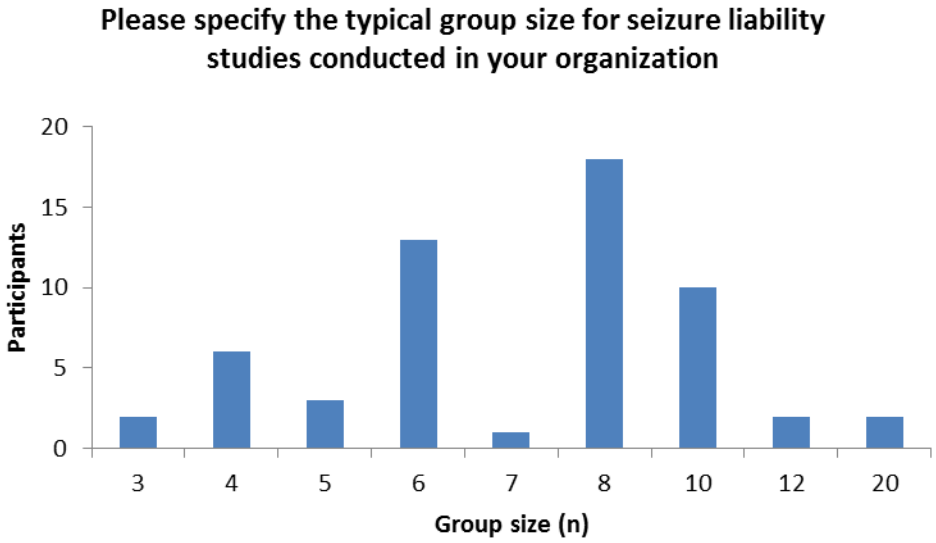


Figure 5: Typical group size in non-clinical seizure liability studies.

Figure 6. Species used in seizure liability studies.

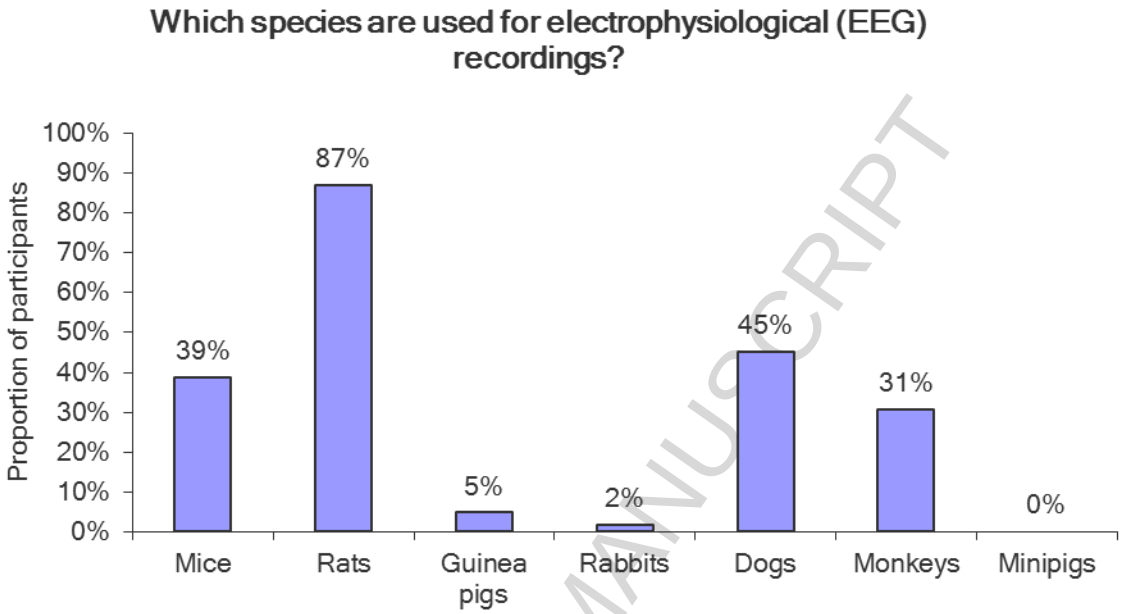


Figure 7. Species used in juvenile non-clinical CNS safety studies.

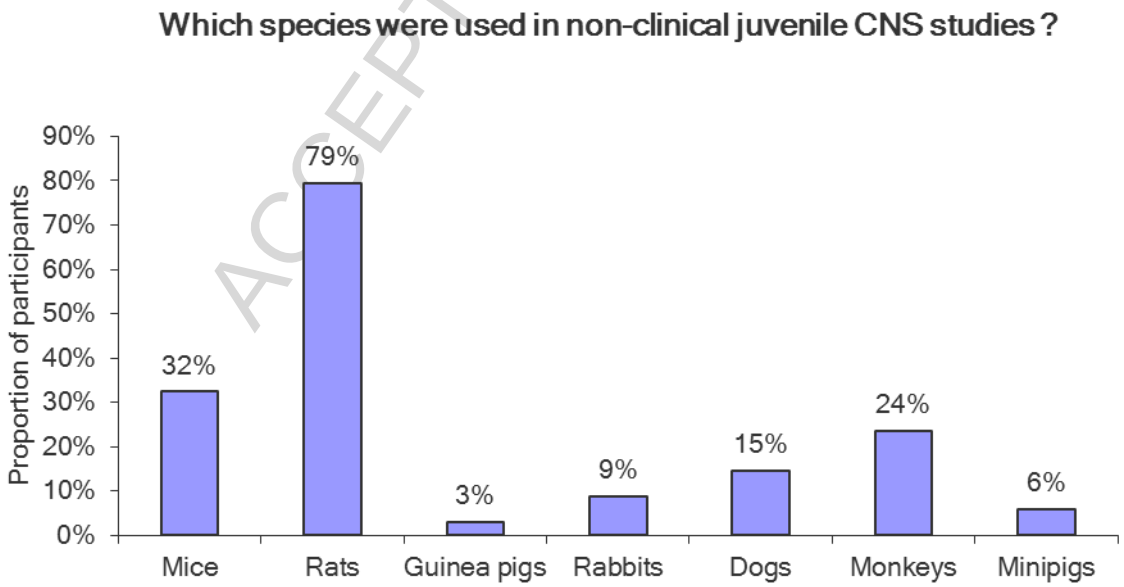
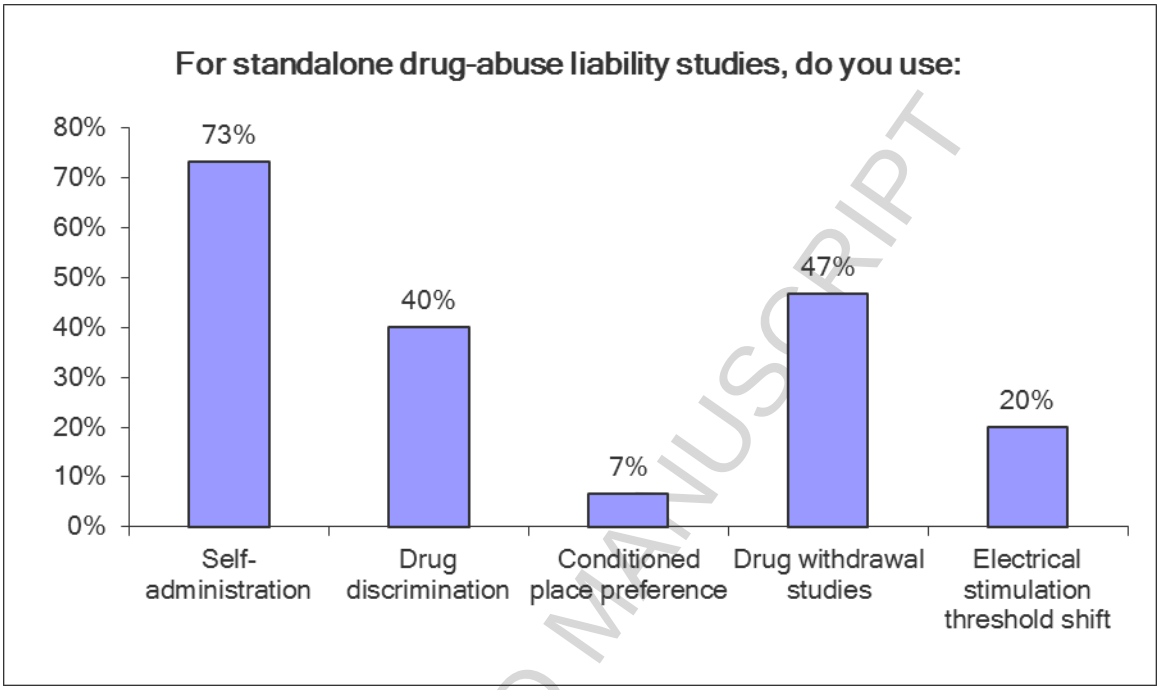


Figure 8. Study types for drug abuse liability studies.



Tables

Table 1 Species in which FOB/Irwin test are conducted

In which species are FOB/Irwin conducted?*		
Answer Options	**Response Percent**	**Response Count**
Mice	36%	49
Rats	96%	130
Guinea pigs	1%	1
Rabbits	1%	1
Dogs	22%	30
Monkeys	35%	47
Minipigs	4%	6

* Participants selected all that applied.

Table 2 Study design characteristics when conducting FOB Assessments

Please define FOB/Irwin study design used in your organization?*		
Answer Options	**Response Percent**	**Response Count**
Standalone study	73%	118
Added to a toxicology study	54%	87
Evaluation after a single dose administration	59%	95
Three dose levels	65%	106
Dose escalating design (i.e. increasing dose levels to the same animals)	12%	19
Repeated dose administration (i.e. multiple days of treatment at the same dose level followed by FOB/Irwin test)	40%	64

* Participants selected all that applied.

Table 3. Quality standards used for FOB, seizure liability, peripheral nerve safety and drug-abuse liability studies

In your organization, are FOB/Irwin studies typically conducted under GLP?		
Answer Options	**Response Percent**	**Response Count**
Yes	50%	64
No	12%	15
Both GLP and Non-GLP	40%	51
In your organization, are seizure liability studies conducted under GLP?		
Answer Options	**Response Percent**	**Response Count**
Yes	15%	11
No	43%	31
Both GLP and Non-GLP	43%	31
In your organization, are peripheral nerve safety testing studies conducted under GLP?		
Answer Options	**Response Percent**	**Response Count**
Yes	24%	9
No	38%	14
Both GLP and Non-GLP	38%	14
In your organization, are drug abuse liability testing conducted under GLP?		
Answer Options	**Response Percent**	**Response Count**
Yes	22%	4
No	44%	8
Both GLP and non-GLP	33%	6

Table 4. Parameters commonly included in FOB/Irwin studies

Which tests were commonly included in FOB/Irwin studies at your organization?*		
Answer Options	**Response Percent**	**Response Count**
We follow the Modified Irwin test	79%	95
Rectal temperature	67%	81
Open field (Gait/coordination, Number of urination/defecation, Number of rearings)	73%	88
Pupillary light response	55%	66
Maze/cognitive tests	9%	11
Landing foot splay	41%	49
Rotarod	26%	32
Grip strength	60%	73

* Participants selected all that applied.

Table 5. Seizure liability study designs

Which study design have you used for seizure liability testing?*		
Answer Options	**Response Percent**	**Response Count**
We include a vehicle control group	87%	65
We include test article groups only	21%	16
We include a PTZ threshold test	61%	46
We use an electroshock seizure (ECS) threshold test	20%	15
We include a positive control group (i.e. proconvulsant) in the PTZ or ECS threshold test	53%	40
We use repeated dose design	33%	25
We collect TK sample, please specify	39%	29
We collect cerebrospinal fluid (CSF)	17%	13
We collect brain tissues	28%	21
I do not know	8%	6

* Participants selected all that applied.

Table 6. Species used for seizure liability studies

Which species were used for seizure testing?*		
Answer Options	**Response Percent**	**Response Count**
Mice	47%	35
Rats	91%	67
Guinea pigs	1%	1
Rabbits	0%	0
Dogs	37%	27
Monkeys	24%	18
Minipigs	0%	0

* Participants selected all that applied.

Table 7. Electrophysiological methodologies for seizure liability studies

Which electrophysiological methodologies were used for seizure testing?*		
Answer Options	**Response Percent**	**Response Count**
Surface EEG (on skin)	44%	30
Implanted EEG	59%	40
Surface EMG (on skin)	12%	8
Implanted EMG	27%	18
Telemetry	50%	34
Video	57%	39
Spectral analysis of EEG (qEEG)	31%	21
Automated seizure detection	25%	17
Manual seizure detection	62%	42

* Participants selected all that applied.

Table 8. Parameters evaluated in polysomnography (sleep) studies.

Which parameters were evaluated in non-clinical sleep studies?*		
Answer Options	**Response Percent**	**Response Count**
EEG (electroencephalography)	67%	20
EMG (electromyography)	43%	13
EOG (electro-oculography)	23%	7
Video monitoring	53%	16
Activity pattern (beam break, accelerometer, open field)	37%	11
Body temperature	43%	13

* Participants selected all that applied.

Table 9. Species used for peripheral nerve safety studies.

Which species were used in peripheral nerve safety testing studies?*		
Answer Options	**Response Percent**	**Response Count**
Mice	31%	11
Rats	72%	26
Guinea pigs	0%	0
Rabbits	8%	3
Dogs	31%	11
Monkeys	31%	11
Minipigs	0%	0

* Participants selected all that applied.

Table 10. Parameters used for peripheral nerve safety studies.

Which parameters were evaluated in peripheral nerve safety testing studies (Select all that apply)?*		
Answer Options	**Response Percent**	**Response Count**
Nerve conduction velocity	68%	23
Grip strength	32%	11
Motor coordination	44%	15
Nociception	32%	11
Allodynia (e.g. von Frey meter)	38%	13
Histopathology	62%	21

* Participants selected all that applied.

Table 11. *In vitro* and other techniques for CNS safety assessment

Have you used any of the following in vitro techniques in the last 5 years?*			
Answer Options	**Yes**	**No**	**Response Count**
Hippocampal brain slice electrophysiology: seizure liability	30	75	105
Hippocampal brain slice electrophysiology: long-term potentiation	17	83	100
Brain slice electrophysiology – other brain region	13	84	97
Neuronal cell line	27	74	101
Neuronal/glial co-culture	17	81	98
Stem cell derived neurons	14	82	96
In vitro blood-brain barrier	20	79	99
Larval zebrafish seizure assay	15	84	99
Larval zebrafish ototoxicity assay	3	94	97

* Participants selected all that applied.